PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P25973WO-KMN	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/GB2004/004274	International filing date *(day/month/year)* 08.10.2004	Priority date *(day/month/year)* 09.10.2003
International Patent Classification (IPC) or national classification and IPC A61K35/74, A61K39/095, C12N1/00		
Applicant HEALTH PROTECTION AGENCY et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 15 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.05.2005	23.03.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004274

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-24 as originally filed

   Claims, Numbers

   1-43 received on 09.12.2005 with letter of 08.12.2005

   Drawings, Sheets

   1/5-5/5 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 19 (completely), 35,42,43 (all partially)

because:

☒ the said international application, or the said claims Nos. 35,43 (concerning industrial applicability) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 19 (completely), 42,43 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-18,20-41 (all completely), 42,43 (all partially) .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,4,21,22,39,40
	No:	Claims	1,2,5-18,20,23-38,41-43
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18,20-43
Industrial applicability (IA)	Yes:	Claims	1-18,20-34,36-42
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Amendments

The amendments of the claims filed with the letter dated 8 December 2005 meet the requirements of Article 34(2)(b) PCT.

The following documents (D) are referred to in this communication:

D1: PEETERS C C A M et al. Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine. VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, 1996, vol. 14, pages 1009-1015

D2: Boulton I C et al. Neisserial binding to CEACAM1 arrests the activation and proliferation of CD4+ T lymphocytes. Nature immunology, 2002, vol. 3, pages: 229 - 236

D3: Cohen M S et al. Human experimentation with *Neisseria gonorrhoeae*: progress and goals. The Journal of infectious diseases, 1999, vol. 179 Suppl 2 , pages S375 - S379

D4: NORMARK STAFFAN ET AL: "Gonococci cause immunosuppression by engaging a coinhibitory receptor on T lymphocytes." NATURE IMMUNOLOGY. MAR 2002, vol. 3, no. 3, pages 210-211

D5: DEHIO C ET AL: "The role of neisserial Opa proteins in interactions with host cells." TRENDS IN MICROBIOLOGY, vol. 6, no. 12, December 1998, pages 489-495

D7: VAN PUTTEN J P ET AL: "Binding of syndecan-like cell surface proteoglycan receptors is required for *Neisseria gonorrhoeae* entry into human mucosal cells." THE EMBO JOURNAL, vol. 14, no. 10, 15 May 1995, pages 2144-2154

D8: VAN DER LEY P ET AL: "Construction of Neisseria meningitidis strains carrying multiple chromosomal copies of the porA gene for use in the production of a multivalent outer membrane vesicle vaccine" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 13, no. 4, 1995, pages 401-407

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Regarding Invention I:

Claims 35,43 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Regarding Invention II:

Claims 35,43 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

This Authority considers that there are 3 inventions covered by the claims indicated as follows:

I: Claims 1-12,20-27,35-43 (all partially), 13-17 (all completely) directed to a method of selecting or preparing microorganisms, compositions or vaccines that are free of OPA for treatment.

II: Claims 1-12,20-27,35-43 (all partially), 18,28-34 (all completely) directed to a method of selecting or preparing microorganisms, compositions or vaccines that contain OPA which does not bind to CEACAM1 for treatment.

III: Claims 19 (completely), 42,43 (all partially) directed to a composition comprising *Neisseria* outer membrane vesicles which comprise an antagonist which inhibits binding of Opa to CEACAM1.

The reasons for which the inventions do not meet the requirements of unity of invention as defined in Rule 13.1 PCT, are as follows:

1 The concept underlying the present application is that avoidance of Opa/CEACAM1 interaction increases the immunogenicity of a meningococcal vaccine.

The latter concept, however, has been disclosed already by document D2, which discloses that the interaction between Opa and CEACAM1 has an immunosuppressive effect on $CD4^+$ T lymphocytes (the whole document) while Opa-negative organisms have not (Fig. 3). Document D2 also discloses to extend the findings of the in vitro studies to in vivo studies (page 234, right-hand column, paragraph 3), and cites in this context D3, which discloses human experimentation with *Neisseria gonorrhoeae* for vaccine testing (the whole document).

Document D4, which is a commentary about D2, also stresses the point to compare the immune response caused by CEACAM1-binding bacteria to that caused by gonococci that do not bind CEACAM1 (the whole document, in particular page 211, right-hand column, last paragraph).

2 In the light of the prior art, it can be concluded that the inventions listed above are not so linked by common inventive concept (Rule 13.1 PCT).

3 In the applicant's/representative's letter dated 22 February 2006 examination is requested for inventions I and II.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Regarding Invention I:

4 D1 discloses a vaccine containing PorA, PorB and Rmp, i.e. a vaccine free of Opa (e.g. page 1009, left-hand column; page 1010, left-hand column, paragraph 1) and thus implicitly a vaccine that is free of Opa that binds CEACAM1.
D1 also discloses OMV-based vaccines in which bactericidal antibodies are induced by PorA-positive but not by PorA-negative mutants (page 1014, left-hand column, last paragraph), showing PorA as the most important component of such a vaccine.
D1 describes further that the deletion of Opa expression increases the purity of the vaccine (page 1014, right-hand column) and cites in this respect reference 12 (document D8).
D1 also discloses a method of selecting such a vaccine as it tests and compares several different vaccines (page 1009, left-hand column; page 1010, left-hand column; page 1014, right-hand column).

D8 discloses regarding the production of a multivalent outer membrane vesicle vaccine that the removal of unnecessary or unwanted outer membrane components, such as opa - e.g. by "mutation" i.e. by replacing opa by another gene (page 402, right-hand column, last paragraph - page 403, left-hand column) - will improve the range of protection of such a vaccine (abstract; page 401, left-hand column - page 402, left-hand column, paragraph 2; page 402, right-hand column, last paragraph-page 403, left-hand column); method to determine the presence of Opa (Fig. 2).

Thus, D1 discloses a vaccine free of Opa and the previous objections are repeated:

In the light of the disclosure of D1, the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,2,6-17,20,24-27,35-38,42,43 is not new in the sense of Article 33(2) PCT.

Document D1 discloses (the references in parentheses applying to this document): meningococcal outer membrane vesicle vaccine, the purity of which is improved by deleting Opa expression (the whole document, e.g. page 1014, right-hand column, paragraph 1) and carrier (page 1010, left-hand column, paragraphs 1-2).

D1 also discloses a vaccine that induces antibacterial IgG antibodies (e.g. Fig. 1). The production of IgG in a host requires isotype switching, which is triggered upon activation of CD4+ T cells. Thus, the vaccine disclosed in D1 may be considered as being free of protein that suppresses activation or proliferation of CD4+ T cells. The preparation of OMV as described in D1 (page 1010, left-hand column, paragraph 2) is generally carried out at a number of starting bacteria that is higher than 1000.

5 D2 discloses to extend the findings of the in vitro studies to in vivo studies (page 234, right-hand column, paragraph 3), and cites in this context D3, which discloses human experimentation with Neisseria gonorrhoeae for vaccine testing (reference 55 in D2). D2 also discloses methods to select Opa variants that bind CEACAM1 by citing reference 14 (page 229, right-hand column, paragraph 2 - page 230, left-hand column, first paragraph).

Thus, the previous objections are repeated:

In the light of the disclosure of D2 (see above), the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,2,5-17,20,23-25,35-38,41-43 is not new in the sense of Article 33(2) PCT.

6 Claims 3,4,21,22,39,40 meet the requirements of Article 33(2) PCT because their subject-matter was not disclosed in the available prior art.

7 Even if the novelty objections above could have been overcome, the subject-matter of invention I still lacks an inventive step (Article 33(3) PCT).

The document D1 is regarded as being the closest prior art to the subject-matter of invention I, and makes the disclosures as stated above.

The subject-matter of invention I therefore differs from document D1 in that: the vaccine is Opa free not because it is seen as an impurity but because it is seen as a protein with immunosuppressive properties.

The problem to be solved by the present invention may therefore be regarded as

providing a further vaccine for the treatment of prevention of disease caused by Gram negative bacteria.

The solution to this problem proposed by invention I consists of the provision of an Opa-free vaccine.

The solution proposed by invention I of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
D1 describes Opa as not only being dispensable but even as being an impurity for a meningococcal vaccine and discloses Opa-free vaccines - which by itself already leads to the novelty objections as outlined above.
D2 discloses the immunosuppressive effect of Opa-mediated ligation of CEACAM1.
Thus, the skilled person, knowing that Opa is dispensable (D1) and suppresses the immune system (D2) would have chosen a vaccine that is deleted in Opa expression, i.e. that is Opa free, as described for example in D1.

In claims 3,4,21,22,39,40 slight constructional changes in the subject-matter disclosed in D1 or D2 are defined which come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

Thus, the subject-matter of invention I lacks an inventive step (Article 33(3) PCT).

8 The subject-matter of claims 1-17,20-27,36-42 is susceptible of industrial application (Article 33(4) PCT).

9 For the assessment of the present claims 35,43 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Regarding Invention II:

10 In the light of the disclosure of D1 (see above), the subject-matter of claims 1,2,6-17;20,24-27,35-38,42,43 is not new in the sense of Article 33(2) PCT.

11 In the light of the disclosure of D2 (see above), the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,2,5-12,20,23-25,35-38,41-43 is not new in the sense of Article 33(2) PCT.

12 In the light of the disclosure of D7, the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 11,12,18,28-34 is not new in the sense of Article 33(2) PCT.

Document D7 discloses (the references in parentheses applying to this document): *Neisseria gonorrhoeae* strain MS11 recombinants that produce Opa_{30} or Opa_{50} (page 2151, right-hand column, paragraph 4; Fig. 8), outer membranes which are only subsequently solubilized (page 2153, left-hand column, last paragraph; Fig. 9), E. coli carrying the opa_{50} gene (page 2151, right-hand column, paragraph 4), Opa-specific antibody 4B12/C11 (page 2153, right-hand column, first paragraph).

Concerning the subject-matter of claim 11 it should be mentioned that a product is not rendered novel by the fact that it is produced by a potentially new process (PCT Guidelines Appendix A5.26[1], 2004).

Note: Opa_{30} and Opa_{50} do not bind CD66a (e.g. D5: Table 1).

13 In claims 3,4,21,22,39,40 slight constructional changes in the subject-matter disclosed in D1 and/or D2 are defined which come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen.

Thus, the subject-matter of claims 3,4,21,22,39,40 lacks an inventive step (Article 33(3) PCT).

14 Note: The core of invention II, i.e. 'use of neisseria outer membrane vesicles which contain Opa that does not bind CEACAM1 but which are substantially free of Opa that binds CEACAM1 for the manufacture of a vaccine for use in treatment or prevention of meningococcal disease' (modified according to page 3 of the present application) appears to be inventive (Article 33(3) PCT) when starting from D2 as the closest prior art.

However, the present set of claims is not limited to the core of invention II, partially because of the use of broad terms that embrace the subject-matter of invention I and/or prior art, and consequently result in the objections as outlined above.

15 The subject-matter of claims 1-12,18,20-34,36-42 is susceptible of industrial application (Article 33(4) PCT).

16 For the assessment of the present claims 35,43 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI
Certain documents cited

Certain published documents

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO2004014417 A	19 Feb 2004	31 July 2003	5 March 2003

Re Item VIII

Certain observations on the international application

Regarding Invention I:

17 The application does not meet the requirements of Article 6 PCT, because claim 38 is not clear. It is not clear what weight% of Opa the predetermined level has.

18 The application does not meet the requirements of Article 6 PCT, because claim 42 is not clear. It is not clear in comparison to which medicament and to which extent the immune stimulation is enhanced by the medicament of claim 42.

19 The expression "incorporated herein" on page 10, line 1 should have been deleted from the description. If matter in the documents referred to is essential to satisfy requirements of Art. 5 PCT, then this matter should be expressly incorporated into the description.

20 The term "substantially" used in claims 1,2,9,12,13,42 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

Regarding Invention II:

21 Claims 18,28,34 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved, i.e. as mimic, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

22 The application does not meet the requirements of Article 6 PCT, because claim 38 is not clear. It is not clear what weight% of Opa the predetermined level has.

23 The application does not meet the requirements of Article 6 PCT, because claim 42 is

not clear. It is not clear in comparison to which medicament and to which extent the immune stimulation is enhanced by the medicament of claim 42.

24 The subject-matter of claim 11 is defined in the term of process for its preparation ('product-by-process' claims).
Claims for products, defined in terms of a process of manufacture, are considered as meeting the requirements of Article 6 PCT provided there is no other information available in the application, which could enable the applicant to define the product satisfactorily by reference to its composition, structure or some other testable parameter.
In consequence, the conditions to define a product by its process of production are that there are no other parameters available for a further definition of the product, which is not the case here.

25 The expression "incorporated herein" on page 10, line 1 should have been deleted from the description. If matter in the documents referred to is essential to satisfy requirements of Art. 5 PCT, then this matter should have been expressly incorporated into the description.

26 The term "substantially" used in claims 1,2,9,12,42 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

CLAIMS

1. A method of selecting a microorganism, composition, vaccine or vaccine component, for use in treatment or prevention of disease caused by Gram negative bacteria, comprising determining whether said microorganism, composition, vaccine or vaccine component is substantially free of Opa that binds *CEACAM1*.

2. A method according to Claim 1, wherein said microorganism, composition, vaccine or vaccine component is selected to be substantially free of Opa that binds *CEACAM1* or is modified so as to be substantially free of Opa that binds *CEACAM1*.

3. A method according to Claim 1 or 2, wherein said determining comprises exposing said microorganism, composition, vaccine or vaccine component to a *CEACAM1-Fc* fusion protein in an ELISA assay.

4. A method according to Claim 3, wherein said determining further comprises contacting said microorganism, composition, vaccine or vaccine component with an Opa-specific monoclonal antibody.

5. A method according to Claim 1 or 2, wherein said determining comprises characterising the interaction between said microorganism, composition, vaccine or vaccine component and *CEACAM1* by ELISA.

6. A method according to any preceding claim, wherein said Gram negative bacteria is selected from *Neisseria*, *Moraxella*, *Kingella*, *Acinetobacter*, *Brucella*, *Bordetella*, *Porphyromonas*, *Actinobacillus*, *Borelia*, *Serratia*, *Campylobacter*, *Helicobacter*, *Haemophilus*, *Escherichia*, *Legionella*, *Salmonella*, *Pseudomonas* and *Yersinia*.

7. A method according to Claim 6, wherein said microorganism is a Neisseria.

8. A method according to Claim 7, wherein the bacterium is *Neisseria meningitidis*.

9. A method according to Claim 7 or 8, wherein said Neisseria is selected to be substantially free of Opa that binds *CEACAM1* or is modified so as to be substantially free of Opa that binds *CEACAM1*.

10. A method according to Claim 9, wherein said Neisseria is modified by mutation.

11. A microorganism, composition, vaccine or vaccine component obtained according to any of Claims 1 to 10.

12. A population of Gram negative bacteria, being 1,000 or more in number, substantially free of bacteria expressing Opa that binds *CEACAM1*.

13. A composition, comprising neisseria outer membrane vesicles, wherein the vesicles are substantially free of Opa.

14. A composition according to Claim 13, wherein the Opa content of the vesicles is reduced by at least a factor of 10 compared with the Opa content of OMVs obtained from normal neisseria.

15. A composition according to Claim 13 or 14 wherein Opa represents 1% or less by weight of the total protein content of OMVs.

16. A composition according to any of Claims 13-15, further comprising a pharmaceutically acceptable carrier.

17. A composition according to any of Claims 13-16, for vaccination.

18. A composition, comprising neisseria outer membrane vesicles, wherein the vesicles comprise a protein which:-

is antigenic,

elicits production of antibodies which bind to Opa, and

does not bind to *CEACAM1*,

wherein the protein is a mutant or fragment or variant or derivative or mimic of Opa.

19. A composition, comprising neisseria outer membrane vesicles, wherein the vesicles comprise an antagonist which inhibits binding of Opa to *CEACAM1*.

20. A method of preparing a composition for use as or in manufacture of a vaccine, the method comprising:-
(a) obtaining a Gram negative bacterium;
(b) determining whether the bacterium expresses an Opa protein that binds to *CEACAM1*;
(c) if the bacterium expresses the Opa protein, discarding the bacterium and repeating steps (a) to (c);
(d) retaining the bacterium if it does not express the Opa protein; and
(e) preparing a composition comprising the retained bacterium of step (d).

21. A method according to Claim 20, wherein said determining comprises exposing said Opa protein to a *CEACAM1-Fc* fusion protein in an ELISA assay.

22. A method according to Claim 21, wherein said determining further comprises contacting said Opa protein with an Opa-specific monoclonal antibody.

23. A method according to Claim 20, wherein said determining comprises characterizing the interaction between said Opa protein and *CEACAM1* by ELISA.

24. A method according to any of Claims 20 to 23, wherein the bacterium is a *Neisseria*.

25. A method according to Claim 24, wherein the bacterium is *Neisseria meningitidis*.

26. A method according to any of Claims 20 to 25, comprising retaining a bacterium which expresses a mutant or variant or fragment or derivative of Opa, wherein the mutant or variant or fragment or derivative does not bind to *CEACAM1*.

27. A method according to any of Claims 20 to 26, comprising preparing an outer membrane vesicle from the retained bacterium.

28. A method of obtaining a mutant or variant or fragment or derivative or mimic of Opa, the method comprising:-
(a) obtaining a Gram negative bacterium;
(b) carrying out mutagenesis on the bacterium;
(c) determining whether the bacterium expresses a mutant or fragment or variant or derivative or mimic of an Opa protein that does not bind to *CEACAM1*;
(d) isolating the mutant or variant or fragment or derivative or mimic, wherein the mutant or variant or fragment or derivative or mimic does not bind to *CEACAM1*.

29. A method according to Claim 28, wherein said determining comprises exposing said mutant or variant or fragment or derivative or mimic of Opa to a *CEACAM1-Fc* fusion protein in an ELISA assay.

30. A method according to Claim 29, wherein said determining further comprises contacting said mutant or variant or fragment or derivative or mimic of Opa with an Opa-specific monoclonal antibody.

31. A method according to Claim 28, wherein said determining comprises characterizing the interaction between said mutant or variant or fragment or derivative or mimic of Opa and *CEACAM1* by ELISA.

32. A method according to any of Claims 28 to 31, wherein the bacterium is a *Neisseria*.

33. A method according to Claim 32, wherein the bacterium is *Neisseria meningitidis*.

34. A method according to any of Claims 28 to 33, further comprising:-
(e) raising an antibody to the mutant or fragment or variant or derivative or mimic; and
(f) determining whether the antibody also binds to an Opa protein that binds to *CEACAM1*.

35. A method of treatment or prevention of disease, comprising administering a microorganism, composition, vaccine or vaccine component of Claim 11, or a population of Gram negative bacteria of Claim 12 or composition according to any of Claims 13 to 19.

36. A vaccine comprising a microorganism, composition, vaccine or vaccine component of Claim 11, or a population of Gram negative bacteria of Claim 12 or composition according to any of Claims 13 to 19.

37. A method of manufacture or testing of a vaccine, the method comprising:-
(a) obtaining a sample of a vaccine or of a component of a proposed vaccine

against a Gram negative bacteria; and

(b) determining whether the sample contains an Opa protein that binds to *CEACAM1*.

38. A method according to Claim 37, further comprising:-

(c) determining the weight % of the Opa protein, if present, by weight % of total protein content in the vaccine or in the sample; and

(d) rejecting the vaccine or the component if the sample contains the Opa protein, or if the weight% of the Opa protein is above a predetermined level.

39. A method according to Claim 37, wherein said determining comprises exposing said sample to a *CEACAM1-Fc* fusion in an ELISA assay.

40. A method according to Claim 39, wherein said determining further comprises contacting said sample with an Opa-specific monoclonal antibody.

41. A method according to Claim 37, wherein said determining comprises characterizing the interaction between said sample and *CEACAM1* by ELISA.

42. Use of *Neisseria* outer membrane vesicles which (i) are substantially free of Opa, (ii) comprise an Opa protein that does not bind to *CEACAM1*, (iii) comprise a mutant or fragment or derivative of Opa that does not bind to *CEACAM1*, or (iv) comprise an antagonist which inhibits binding of Opa to *CEACAM1*, in manufacture of a medicament for treatment or prevention of meningococcal disease with improved stimulation of immune memory or reduced inhibition of T cell function.

43. A method of treatment or prevention of disease caused by Gram negative bacteria comprising administering a microorganism, composition, vaccine or vaccine component, wherein said microorganism, composition, vaccine or vaccine component (i) is substantially free of Opa, (ii) comprises an Opa protein that does not bind to *CEACAM1*, (iii) comprises a mutant or fragment

or derivative of Opa that does not bind to *CEACAM1*, or (iv) comprises an antagonist which inhibits binding of Opa to *CEACAM1*, and wherein said treatment or prevention involves the removal of suppression of activation or proliferation of CD4+ T-lymphocytes.